

THE BIONOMICS AND LIFE CYCLE OF
Trichodorus christiei

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TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	ii
LIST OF TABLES	vi
LIST OF FIGURES	ix
INTRODUCTION	1
REVIEW OF LITERATURE	2
MATERIALS AND METHODS	6
FIELD EXPERIMENTS	6
Field Experiment 1. Comparison of the effects of 1, 3-dichloropropene 1, 2-dichloropropane (D-D soil fumigant) fumigation with no fumigation on the population of <u>Trichodorus christiei</u> on fall, spring and summer crops	6
Field Experiment 2. A split-plot experiment designed to study the residual effect of three nematicides in the control of <u>Trichodorus christiei</u>	7
Field Experiment 3. The survival of <u>Trichodorus christiei</u> in the field in the absence of a host	9
GREENHOUSE EXPERIMENTS	9
Greenhouse Experiment 1. Effects of four temperatures on the reproductive rate of <u>Trichodorus christiei</u>	9
Greenhouse Experiment 2. A test designed to determine the host status of pangolagrass, <u>Digitaria decumbens Stent.</u> , to the ectoparasitic nematodes <u>Trichodorus christiei</u> and <u>Belonolaimus longicaudatus</u>	10
Greenhouse Experiment 3. The interrelationship of population levels of <u>Trichodorus christiei</u> and <u>Belonolaimus longicaudatus</u> in the greenhouse utilizing different levels of initial inocula	12

Greenhouse Experiment 4. A comparison of the reproductive rate of 25 <u>Trichodorus christiei</u> in D-D fumigated soil with that in unfumigated soil	12
Greenhouse Experiment 5. Investigation into the possible predaceous habit of three free-living nematodes and an oligochaete on <u>Trichodorus christiei</u>	13
Greenhouse Experiment 6. The effect of different kinds of potting containers on the reproduction of <u>Trichodorus christiei</u>	14
LABORATORY EXPERIMENTS	15
Laboratory Experiment 1. The life cycle of <u>Trichodorus christiei</u> in a constant temperature chamber set at 80°F with a 12-hour photoperiod	15
Laboratory Experiment 2. Mode of molting by <u>Trichodorus christiei</u>	17
Laboratory Experiment 3. Determination of the reproductive potential of <u>Trichodorus christiei</u>	17
Laboratory Experiment 4. Population dynamics of <u>Trichodorus christiei</u> as affected by original numbers. .	17
Laboratory Experiment 5. An investigation into the possibility of a stage resistant to D-D fumigation in the life cycle of <u>Trichodorus christiei</u>	18
Laboratory Experiment 6. Survival of <u>Trichodorus christiei</u> in the absence of a host at 80°F	19
Laboratory Experiment 7. Morphological studies on <u>Trichodorus christiei</u>	20
RESULTS AND DISCUSSION	21
FIELD EXPERIMENTS	21
Field Experiment 1	21
Field Experiment 2	30
Field Experiment 3	36
GREENHOUSE EXPERIMENTS	36

	Page
Greenhouse Experiment 1	37
Greenhouse Experiment 2	38
Greenhouse Experiment 3	41
Greenhouse Experiment 4	46
Greenhouse Experiment 5	46
Greenhouse Experiment 6	49
LABORATORY EXPERIMENTS	51
Laboratory Experiment 1	51
Laboratory Experiment 2	55
Laboratory Experiment 3	58
Laboratory Experiment 4	58
Laboratory Experiment 5	61
Laboratory Experiment 6	66
Laboratory Experiment 7	67
THE BUILD-UP OF <u>TRICHODORUS CHRISTIEI</u>	69
SUMMARY	71
LITERATURE CITED	74
BIOGRAPHICAL SKETCH	77

LIST OF TABLES

Table		Page
1. Crops Used in Comparing D-D Fumigation with No Fumigation		7
2. Nature and Source of the Three Soils Used in the Study of the Host Status of Pangolagrass to <u>Trichodorus christiei</u> and <u>Belonolaimus longicaudatus</u>		10
3a. Numbers of <u>Trichodorus christiei</u> Found in D-D Fumigated Plots Compared to Those in Unfumigated Plots. (a) Fall Application		23
3b. Numbers of <u>Trichodorus christiei</u> Found in D-D Fumigated Plots Compared to Those in Unfumigated Plots. (b) Spring Application.		24
3c. Numbers of <u>Trichodorus christiei</u> Found in D-D Fumigated Plots Compared to Those in Unfumigated Plots. (c) Summer Application.		25
4. The Effect of Sorghum Root-depth on Populations of <u>Trichodorus christiei</u> at Harvest		27
5. Increase in Cabbage Yield and Numbers of <u>Trichodorus christiei</u> Following an Application of D-D Fumigant at 30 Gal per Acre		29
6a. A Comparison of the Effects Certain Nematicides Have On the Build-up of <u>Trichodorus christiei</u> on 'Funks' Hybrid Field Corn		32
6b. A Comparison of the Residual Effects of a Split Application of Certain Nematicides on the Build-up of <u>Trichodorus christiei</u> on Cabbage		33
6c. Effect of Nematicide Treatments on Total Number and Weight of Cabbage Heads on Three Harvest Dates.		34
6d. A Comparison of the Residual Effects Certain Nematicides Have on the Build-up of <u>Trichodorus christiei</u> On Unfumigated 'Gold Cup' Sweet Corn.		35

Table		Page
7.	A Comparison of the Survival of <u>Trichodorus christiei</u> in the Presence and Absence of Organic Matter (O.M.) Under Clean Cultivation in the Field	36
8.	The Effect of Temperature on the Reproductive Rate of 25 <u>Trichodorus christiei</u> on Sweet Corn and Tomato After 8 Weeks	37
9.	Plant Parasitic Nematode Populations in 100 ml of Soil From Under Pangolagrass Followed by Sweet Corn and Vice Versa Maintained in the Greenhouse for 3 Months	39
10.	Total Dry Weight (Grams) of Roots From 5 Replicates of Pangolagrass and Sweet Corn After 3 Months in Association With <u>Trichodorus christiei</u> and <u>Belonolaimus longicaudatus</u>	40
11.	The Interrelationship of Two Ectoparasitic Nematodes At Different Rates of Initial Inoculum While Feeding on Sweet Corn in 6-inch Plastic Pots.	42
12.	Effect of <u>Trichodorus christiei</u> and <u>Belonolaimus longicaudatus</u> on the Dry Weights (Grams) of the Second Crop of Sweet Corn. The Corn was Harvested 45 Days After Replanting in Pots in Which the Nematodes had Previously Been Established.	43
13.	The Reproductive Rate of <u>Trichodorus christiei</u> in D-D Fumigated Soil and Unfumigated Soil in Greenhouse Pots . .	46
14.	Effects of Oligochaetes on Number of <u>Trichodorus christiei</u> After 8 Weeks.	47
15.	Effects of Three Possible Predaceous Nematodes on Populations of <u>Trichodorus christiei</u>	48
16.	Effect of <u>Aporcelaimellus obscurus</u> on Established Populations of <u>Trichodorus christiei</u>	48
17.	Numbers of <u>Trichodorus christiei</u> in 100 ml of Soil From Four Different Containers Using Sweet Corn as a Host After A Period of 8 Weeks.	49
18.	Study of the Life Cycle of the Offspring of 10 Gravid Female <u>Trichodorus christiei</u> at 80°F While Feeding on 'Homestead 24' Tomato	52
19.	Reproductive Rate of Individual Specimens of <u>Trichodorus christiei</u> While Feeding on 'Homestead 24' Tomato Seedlings Growing at A Constant Temperature of 80°F	59

Table		Page
20a.	Reproductive Rates of <u>Trichodorus christiei</u> at Different Rates of Infestation Maintained at 80°F.	60
20b.	Average Weights of 'Homestead 24' Tomatoes Inoculated with <u>Trichodorus christiei</u>	60
21a.	Effects of D-D Fumigation on <u>Trichodorus christiei</u> , <u>Belonolaimus longicaudatus</u> and <u>Hoplolaimus galeatus</u> in Soil Contained in Pint Jars	64
21b.	Effects of D-D Fumigation on a Pure Population of <u>Trichodorus christiei</u> in Soil Contained in Pint Jars	65
22.	Survival of <u>Trichodorus christiei</u> in the Absence of a Host in a 6-inch Pot at 80°F	67

LIST OF FIGURES

Figure	Page
1. Roots of a sweet corn plant showing stubby-root symptoms caused by <u>Trichodorus christiei</u>	26
2. Comparison of sweet corn seedling growth on unfumigated Leon fine sand soil with that fumigated with D-D soil fumigant applied at 30 gal per acre	28
3. The effect of <u>Belonolaimus longicaudatus</u> on the growth of sweet corn 45 days after replanting in pots initially inoculated with (left to right) 250, 100, 50 and 25 specimens	44
4. Growth of sweet corn 45 days after replanting in pots initially inoculated with (left to right) 250, 100, 50 and 25 <u>Trichodorus christiei</u>	45
5. Root growth of sweet corn after 8 weeks in the greenhouse in a clay pot, glazed crock, and plastic pot	50
6. Stages in the life cycle of <u>Trichodorus christiei</u> at (10X); (a) egg, (b) first larval stage, (c) second larval stage, (d) third larval stage, (e) fourth larval stage, and (f) adult female	53
7. Variation in size between adults of <u>Trichodorus christiei</u> collected from two locations near Sanford, Florida	54
8. Break in the old cuticle prior to molting of its "hood" by <u>Trichodorus christiei</u>	56
9. A molting specimen of <u>Trichodorus christiei</u> . Note the absence of the onchiostyle in the "hood"	57
10. Characteristic position of <u>Trichodorus christiei</u> while feeding; i.e., the head at right angles to the cell upon which it is feeding.	62
11. <u>Trichodorus christiei</u> feeding upon the root-cap of tomato in agar	63
12. Water mount of a live <u>Trichodorus christiei</u> recovered from soil with a moisture level of 1.7 per cent	68

INTRODUCTION

The nematode genus Trichodorus was erected in 1913 by Cobb (11) when he described the species Trichodorus obtusus. Micoletzky (22) transferred Dorylaimus primitivus de Man, 1880, to the genus Trichodorus and placed T. obtusus in synonymy. The genus received little further mention in the literature until 1951 when Christie and Perry (8) reported that an undescribed species of Trichodorus was parasitic to several crop plants in Florida. Subsequently this species, described by Allen (2) as Trichodorus christiei, has been recognized as a severe pathogen in many areas of the United States. To date, most of the research on this species deals with host-parasite relationships and control, with little consideration of its biology.

Extremely high population levels of T. christiei are frequently found following the application of soil fumigant nematicides. This unusual population build-up has led to considerable speculations as to the factors responsible for such a phenomenon.

This work on the bionomics and life cycle of T. christiei was initiated as part of a program to attempt an explanation of the population increases of this nematode resulting from soil fumigation.

REVIEW OF LITERATURE

Thorne (33) placed the genus Trichodorus into his family Diphtherophoridae, subfamily Trichodorinae of the superfamily Dorylaimoidea and named T. primitivus (de Man, 1880) Micoletzky, 1922, the type species. Allen (2) published a monograph of the genus Trichodorus and described T. christiei, the species to which Christie and Perry (8) had given the common name of stubby-root nematode. Other species have since been added until now some 32 are recognized.

The feeding habits of T. christiei have been studied by a number of workers (5, 10, 27, 29 and 35). Russell and Perry (29) reported on three general types of feeding on wheat seedlings growing in agar; externally on roots, externally on root hairs, and within root caps. This latter method explains the measurable symptoms commonly found in association with low populations of T. christiei (1 and 29). Christie and Perry (8) proposed that the disease be termed stubby root because through the cessation of longitudinal growth, short, multibranched rootlets are produced.

The effects of temperature on the population numbers of T. christiei have been studied by Malek et al. (20). The greatest numbers after 60 days and 90 days were found at temperatures very close to or at 25°C. The lowest rate of reproduction was at 15°C. At the upper limit, Rhode and Jenkins (26) found that no reproduction took place at 35°C.

Perry (24) observed a rapid build-up of T. christiei following soil fumigation with methyl bromide and other nematicides. Haasis et al. (13), studying 14 species of nematodes associated with decline of woody ornamentals, found that they could control all but T. christiei with 1, 2-dibromo-3-chloropropane. This unique behavior of T. christiei to soil fumigation also has been observed by Martin (21) in Africa. Perry (24) suggested that this nematode has a high reproductive potential. Alhassan and Hollis (1) found that after 3 weeks the per cent increase in numbers of T. christiei was related inversely to the initial inoculum level and to plant damage.

The life cycle of T. christiei has been studied in part. Russell (28) found that eggs hatched 66 to 68 hours after oviposition in vitro, with apparently the first larval stage emerging. Rhode and Jenkins (26) reported that T. christiei completed its life cycle in 16 to 17 days at 30°C and 21 to 22 days at 22°C. Four distinct groups were recovered; in each case specimens were larger at the lower temperature.

Bird (4) demonstrated that both host and geographic origin of T. christiei could influence population density, indicating the possibility of races. The host also had an effect on the size of the nematode; for example, those specimens obtained from lettuce were larger than those from tomato and celery. Rhode and Jenkins (27) reported jimsonweed, asparagus, poinsettia and crotalaria to be the only non-hosts of T. christiei among the 42 species of plants they tested. Rhoades (25) in Florida found the numbers of T. christiei declined greatly during the summer months in plots planted to Crotalaria spectabilis Roth., but persisted in those with weeds.

Population numbers have also been reported to be affected by seasons. Perry (24) found that damage was apparently most severe in a spring crop following a fumigated fall crop. A similar situation was found by Barker and Worf (3) on azaleas in Wisconsin, where no T. christiei were observed during the summer. Hoff and Mai (15) recorded the numbers of T. christiei recovered from onion fields on peat soil at eight depths at intervals of 6 inches down to 4 feet throughout the year in the state of New York. The maximum number was recorded at a depth of 0-6 inches around June 1. T. christiei was not found below 12 inches at any time. Hoff and Mai (15) concluded that "the natural population of T. christiei was maintained in a specific soil and root zone."

Comparing silt clay loam, loam and sandy loam, Thomason (31) found that after twelve weeks the greatest reproduction of T. christiei occurred in the sandy loam. After 19 weeks, however, the sandy loam population was only 1/6 that of the loam. Survival in the absence of a host by T. christiei was also reported as being substantially greater in the loam. According to Christie (7), it seems unlikely that the distribution of T. christiei is greatly influenced by soil type.

Recent agricultural research has stressed biological control. A possible explanation for the rapid return of T. christiei following fumigation may be due to the reduction of predator populations. Working in vitro, Russell (28) found a number of predators which preyed upon T. christiei, the most aggressive of which were the nematode Aphelenchoides winchesi Christie, 1939 and the mite Protolaelaps

bickleyi Bram. Thorne (32) found the remains of Trichodorus primitivus in the gut of Mononchus acutus Cobb, 1917. Chitwood and Oteifa (6) observed oligochaetes feeding upon nematode eggs, while Schaerffenberg (30) demonstrated that enchytraeids controlled Heterodera schachtii Schmidt, 1871 in greenhouse pots. Tardigrades were reported feeding upon Trichodorus aequalis Allen, 1957 by Hutchinson and Streu (16). Esser and Sobers (12) expressed doubt that tardigrades can be utilized in biological control as they are rarely found in abundance. Little is known of the presence of nematicidal fungi in Florida soils and there have been no studies on the effects fumigation have on these fungi.

A survey of the literature revealed no work on the competitive status of Trichodorus when in association with other plant parasitic nematodes. Krusberg and Sasser (18) observed that only small numbers of Meloidogyne sp. and Pratylenchus sp. were present when Hoplolaimus coronatus Cobb, 1923 was abundant. Thorne (34) noted that Xiphinema americanum Cobb, 1913 frequently dominates Pratylenchus penetrans (Cobb, 1917) Filipjev and Stekhoven, 1914 and Pratylenchus minyus Sher and Allen, 1953 when in association with these smaller, slower moving pratylenchs.

MATERIALS AND METHODS

Field Experiments

Field plot experiments involving the use of nematicides were conducted at the Central Florida Experiment Station for the purpose of studying the population build-up of T. christiei following fumigation. Using a randomized block design, plots were laid out on Leon fine sand soil which had been fallow for the past ten years. The dominant plant species prior to plowing was bermudagrass (Cynodon dactylon (L.) Pers.). The area lacked tile which is normally used for drainage and subsurface irrigation; thus, when required, plots were irrigated by an overhead sprinkler system.

Soil samples were taken with a Hoffer soil sampler; eight-in-the-row samples were taken from each plot. With one exception, samples were taken to a depth of 6 inches and the soil placed in polyethylene bags. Individual samples were thoroughly mixed and a 100 ml sub-sample processed by the centrifugal-flotation method (17). Samples were read within 48 hours of sampling. Nematodes were counted in a known area of a syracuse dish under a binocular microscope, and the number per 100 ml of soil was calculated.

Field Experiment 1. --Comparison of the effects of 1,3-dichloropropene 1, 2-dichloropropane (D-D soil fumigant) fumigation with no fumigation on the population of Trichodorus christiei on fall, spring and summer crops.

After bringing the area to seed bed tilth, one half of each block was treated with D-D soil fumigant at the rate of 30 gal per acre

applied with a conventional shank injector at a depth of 6 inches. Two weeks later the plots were fertilized with a 5-5-8 mixture at a rate of 1000 lb per acre, disked and leveled. Two crops were planted, with 5 rows of each per replicate which were 35 ft long by 24 ft wide (Table 1). Data were taken from the 3 rows of each crop, within the border rows.

Table 1. Crops Used in Comparing D-D Fumigation With No Fumigation

Crop	Variety	Planting Date	Harvesting Date
Cabbage	Marion market	10/14/64	2/3/65
Sorghum	Beef builder		
Cabbage	Copenhagen	3/25/65	6/10/65
Sweet corn	Gold cup		
Soybean	Lee	7/19/65	10/20/65
Sorghum x sudan-grass hybrid	Grazer A		
Cabbage	Market topper	11/12/65	2/25/66
Sorghum x sudan-grass hybrid	Grazer A		

Population counts of the nematodes present were made at the end of each month. Yields were taken on a fresh weight basis at harvest.

Field Experiment 2. --A split-plot experiment designed to study the residual effect of three nematicides in the control of *Trichodorus christiei*.

A comparison was made between a single nematicide application prior to a summer crop of 'Funks' hybrid field corn and its effects on

the following fall crop of 'Greenback' cabbage with that of an application prior to both the summer and fall crops on plots 12.5 ft by 14 ft. The experiment was terminated by observing the residual effect of the nematicides on a crop of 'Gold-Cup' sweet corn the following spring.

The treatments included: (1) check, (2) 0-0 diethyl 0-2 pyrazinyl phosphorothioate (zinophos) at 3 lb per acre, (3) 1,2-dibromo-3-chloropropene (Nemagon) at 2 gal per acre, (4) D-D soil fumigant at 15 gal per acre, and (5) D-D soil fumigant at 25 gal per acre.

Prior to fumigation 25 lb of soil containing 100 T. christiei per 100 ml was spread over each plot and disked in. The fumigants were applied by a hand applicator on a 12-inch lattice to a depth of 6 inches 2 weeks before planting. Zinophos was applied as a 10 per cent granular formulation to the soil surface just prior to planting and immediately forked into the soil to a depth of \pm 6 inches. The plots were then fertilized with a 5-5-8 fertilizer mixture at the rate of 1000 lb per acre, disked, leveled and planted.

The plots were cultivated to reduce weed growth and a side dressing of 5-5-8 fertilizer was applied at the rate of 500 lb per acre during the third and sixth week following planting. The insecticide Sevin was applied twice a week at 2 lb per 100 gal of water to control corn earworm. Cabbage looper control was obtained using 1/2 pint of parathion plus 1 quart of toxaphene in 100 gal of water.

Soil samples were taken prior to fumigation, during the growing season, and at harvest.

Field Experiment 3. --The survival of *Trichodorus christiei* in the field in the absence of a host.

A study was made on the effect of a green manure crop incorporated into the soil on the survival of *T. christiei*. Eight field plots 6 ft by 6 ft were planted to a fall crop of sorghum x sudangrass hybrid (variety Grazer A) a known host of *T. christiei*. After 7 weeks all the plots were harvested and the root systems removed. The green matter, at a rate of 17 tons per acre, then was incorporated in one plot of each of the 4 replicates. The experimental area was kept weed-free by hand for the following 16 weeks, during which time the survival of *T. christiei* was recorded in plots with and without organic matter.

Greenhouse Experiments

Greenhouse Experiment 1. --Effects of four temperatures on the reproductive rate of *Trichordorus christiei*.

The study was made in four constant temperature tanks designed by Harrison and Stall (14). Each of the tanks held 16 containers and the tanks were randomly allotted temperatures of 80°, 85°, 90° and 95°F \pm 1.5°F. Leon fine sand soil was fumigated with methyl bromide. After allowing the soil to aerate for a week a 5-5-8 fertilizer mixture was thoroughly mixed with the soil at the rate of 1000 lb per acre and 1100 ml of this soil was used to fill each of 64 plastic containers. Specimens of *T. christiei* were hand picked into distilled water; 25 females (males are very rare) were used to inoculate the soil in each container. At the time of inoculation half of the containers were planted to 'Gold Cup' sweet corn and half to 'Rutgers' tomato. The pots were watered with distilled water as needed. At the end of

8 weeks the experiment was terminated. The soil was thoroughly mixed and a representative sample obtained. A 100 ml sub-sample was processed using the sugar-flotation method to remove the nematodes.

Greenhouse Experiment 2. --A test designed to determine the host status of pangolagrass, Digitaria decumbens Stent., to the ecto-parasitic nematodes Trichodorus christiei and Belonolaimus longicaudatus.

Soil from the Immokolee fine sand series was obtained from three sources (Table 2).

Table 2. Nature and Source of the Three Soils Used in the Study of the Host Status of Pangolagrass to Trichodorus christiei and Belonolaimus longicaudatus.

Origin	pH	O.M. ^a /	Plant parasitic nematodes in 100 ml of soil		
			Stubby-root	Stunt	Ring
"Sterile" ^b /	5.3	4.48%	--	--	--
"Pangola"	7.0	4.32	14	--	--
"Crop"	6.6	2.58	14	32	2

^a/ Chromic acid determination of the organic matter.

^b/ "Sterile" = steam sterilized.

"Pangola" = from under a 3-year-old pangolagrass pasture.

"Crop" = from under sorghum, prior to which there had been 2 crops of tomato.

Twenty 6-inch clay pots were filled with each of the three soils after incorporating the equivalent of 1000 lb per acre of 5-5-8 fertilizer mixture and 500 lb per acre of lime.

The pots were placed in the greenhouse with 5 replicates each of the following host-inoculation combinations in each soil:

	Host	Nematode
(1)	Pangolagrass	<u>T. christiei</u>
(2)	Pangolagrass	<u>B. longicaudatus</u>
(3)	'Gold Cup' sweet corn	<u>T. christiei</u>
(4)	'Gold Cup' sweet corn	<u>B. longicaudatus</u>

Individual sprigs of pangolagrass were planted in each of the designated pots. These sprigs were obtained from one-month-old stem cuttings which had been grown in sterile soil. Three seeds of sweet corn were planted in each indicated pot.

The nematodes were inoculated in water suspensions around the seeds of corn and roots of pangolagrass. The respective treatments received 25 females of T. christiei and 20 females, plus 5 males, of B. longicaudatus. Both species of neamtodes were obtained from a field plot of sorghum.

The pots were maintained for 3 months. At the end of each month the grass was clipped to the first node and a side-dressing of 5-5-8 fertilizer mixture was applied at the rate of 1000 lb per acre to each replicate. On termination of the experiment, fresh weights of roots and tops were recorded, the soil in each replicate was thoroughly mixed, and three composite samples were obtained from each treatment. Nematode-free sprigs of pangolagrass then were planted in those replicates which had grown sweet corn, and sweet corn was planted in those replicates previously growing pangolagrass. These replants were maintained for an additional 3 months and processed in the same manner.

Greenhouse Experiment 3. --The interrelationship of population levels of *Trichodorus christiei* and *Belonolaimus longicaudatus* in the greenhouse utilizing different levels of initial inocula.

The rates of reproduction of pure populations of *T. christiei* and *B. longicaudatus* were compared with a third treatment having these 2 species in combination. The initial inoculum level of each nematode both in the pure and mixed treatments were 25, 50, 100 and 250. Inoculum of *T. christiei* was comprised of females only, while that of *B. longicaudatus* was a 4:1 ratio of females to males.

Plastic pots containing 1,400 ml of autoclaved Leon fine sand, previously fertilized with an equivalent of 1000 lb per acre of 5-5-8 mixture, were planted to 'Gold Cup' sweet corn (3 seeds/pot). Suspensions of the nematode inocula were then washed into the planting holes, and the pots randomized on the greenhouse bench.

After 45 days the soil in each pot was thoroughly mixed and a 100 ml sample taken and processed. Both the corn tops and roots were collected and their dry weights recorded. Following the application of fertilizer the remaining soil was repotted and once again planted to 'Gold Cup' sweet corn. The experiment was terminated 45 days after replanting and similar data were obtained.

Greenhouse Experiment 4. --A comparison of the reproductive rate of 25 *Trichodorus christiei* in D-D fumigated soil with that in unfumigated soil.

The soil used in this experiment was a Leon fine sand which had been fumigated the previous spring with the nematicide D-D (27 gal per acre) prior to planting cantaloupe (*Cucumis melo* L. var *reticulatus* Naud.). A late summer cover crop of *Crotalaria spectabilis* was

planted following the cantaloupe. After the C. spectabilis had been harvested soil was removed from the field, sifted and placed in six 3-gal porcelain crocks. Three of these crocks were fumigated at a depth of 8 inches with 1.21 ml D-D (=30 gal per acre) by means of a pipette fitted with a propippette. The soil temperature at the time of fumigation was 66°F. After waiting 2 weeks, six 6-inch clay pots were filled with fumigated soil and six with the unfumigated soil. At the time of potting a 100 ml sample of soil was processed and the number of nematodes recorded. The pots were seeded with oats (Avena sativa L.), and 25 specimens of T. christiei added to each. At the end of 2 months, the pots were removed and the numbers of T. christiei per 100 ml of soil recorded. The pots were then replanted to 'Gold Cup' sweet corn and allowed to grow for a further 2 months, before once again recording the numbers of nematodes present.

Greenhouse Experiment 5. --Investigation into the possible predaceous habit of three free-living nematodes and an oligochaete on Trichodorus christiei.

The nematode inocula were obtained from the test conducted on the host status of pangolagrass to T. christiei. The specimens were hand picked into distilled water and then poured into 6-inch clay pots containing autoclaved Leon fine sand soil which was then seeded with 'Gold Cup' sweet corn. The design of the treatments applied to each of the three possible predaceous nematodes being tested was as follows with 5 replicates of each:

- (1) 100 T. christiei
- (2) 100 T. christiei + 25 free-living specimens

- (3) 100 T. christiei + 100 free-living specimens
- (4) 25 free-living specimens

The free-living nematodes included Aporcelaimellus obscurus (Thorne and Swanger, 1936) Heyns, 1965; Eudorylaimus simplex (Thorne and Swanger, 1936) Andrassy, 1959 and Mylonchulus parabrachyurus (Thorne, 1924) Andrassy, 1958.

Since oligochaetes are commonly found in unfumigated soil, a test was designed to investigate the effect they might have on the reproduction of T. christiei. The treatments consisted of the following three rates of hand picked inocula replicated five times:

- (1) 100 T. christiei
- (2) 100 T. christiei + 100 oligochaetes
- (3) 100 T. christiei + 500 oligochaetes

The inocula was poured into 6-inch plastic pots containing autoclaved Leon fine sand soil which had been fertilized at the equivalent rate of 1000 lb per acre of 5-5-8 mixture. The host plant used in this experiment was 'Homestead 24' tomato. After planting, the treatments were randomly placed in a growth chamber set at 80°F with a 12-hour photoperiod. Top and root weights were recorded after 8 weeks, and the soil in each pot was thoroughly mixed and a 100 ml sample processed from each.

Greenhouse Experiment 6. --The effect of different kinds of potting containers on the reproduction of Trichodorus christiei.

Clay pots have conventionally been used for determining nematode pathogenicity and reproduction on a host. Frequently seedling roots

which escape nematode damage grow out to the walls of the pot, where they produce a mat. Owing to the porous nature of clay, there are greater moisture fluctuations along the pot walls, producing an environment which is less favorable to nematodes than the center of the pot.

Previous indications were that roots did not mat along the walls of glazed crocks and plastic pots. Experiments were conducted in a greenhouse and growth chamber set at 80°F to determine the environmental effects in four different 6-inch containers on the reproduction of T. christiei. The containers used were as follows:

- (1) Red clay pot; surface irrigated.
- (2) Red clay pot; sub-surface irrigated.
- (3) Glazed crock.
- (4) Plastic pot.

Five containers of each type were filled with 1,350 ml of autoclaved Leon fine sand. Each of the pots was seeded with 3 'Gold Cup' sweet corn seeds, and an inoculum suspension of 25 T. christiei poured over the seeds. In addition to the inoculated pots, 5 replicates of uninoculated pots were set up in the growth chamber as checks.

After 8 weeks the pots were removed and top and root weights recorded. The soil in each pot was thoroughly mixed and a 100 ml sample processed to determine the nematode numbers.

Laboratory Experiments

Laboratory Experiment 1. --The life cycle of Trichodorus christiei in a constant temperature chamber set at 80°F with a 12-hour photoperiod.

Styrofoam cups were cut in half and 50 ml of autoclaved Leon fine sand placed in each. 'Homestead 24' variety of tomato was then sown in the cups and after germination thinned to 4 plants per cup. Two weeks after planting 10 gravid females of T. christiei obtained from a tomato host were poured into a hole adjacent to one of the tomato seedlings in each of 48 cups. From the third to the eighteenth day, 4 cups were removed per day.

The soil was processed using an unpublished technique devised by the late M. B. Linford of the University of Illinois. The soil and nematodes collected from the washings on the 325-mesh screen were poured onto a folded Kimwipe tissue held between two 2-ounce plastic funnels. The funnels were placed on the walls of a syracuse dish in such a manner as to suspend the tissue above the bottom of the dish. After allowing the washings to settle on the tissue (1-2 minutes) the funnels were transferred to a second syracuse dish. The water level in this dish was raised to a level just above that of the tissue. After 6 hours the funnels were transferred to a third syracuse dish and the water level was adjusted as described above. The funnels were removed from this third dish after 2 hours, and the numbers and stages of development of the nematode specimens were recorded.

Specimens of the oldest stage collected each day were placed in a specimen vial filled 1/4 full of distilled water, and the remainder of the offspring were placed in another similar vial. The vials were then transferred to a hot water bath at 50°^OC for 15 minutes, at which time the level in the vials was brought to 1/2, using 2 per cent formalin solution for fixation and preservation.

Laboratory Experiment 2. --Mode of molting by Trichodorus christiei.

Specimens of T. christiei were studied while molting in water and in 3/4 per cent water agar contained in the top half of 60 x 15 mm plastic culture dishes. After picking the nemas into the agar, the surface of the agar was covered with a disk of plastic sheet to prevent drying and facilitate studies with the aid of a compound microscope. Other specimens were fixed at various stages throughout molting by placing them in 1 per cent formalin at 50°C. These were then mounted on Cobb aluminum slide holders to facilitate study from both sides with the aid of oil immersion objectives.

Laboratory Experiment 3. --Determination of the reproductive potential of Trichodorus christiei.

Styrofoam cups were prepared as above, but in this experiment 48 cups were inoculated with individual fourth stage larvae and placed in a growth chamber set at 80°F with a 12-hour photoperiod. At the end of 16, 17, 18, 19 and 38 days, 8 cups were processed using Linford's funnels. Counts were made of the numbers of each life cycle stage of T. christiei present in each cup. This experiment was duplicated.

Laboratory Experiment 4. --Population dynamics of Trichodorus christiei as affected by original numbers.

The effects of initial inocula numbers on the reproductive rate of T. christiei were studied in a constant temperature chamber set at 80°F with a photoperiod of 12 hours. The treatments included hand picked inocula of 25, 100 and 400 females of T. christiei inoculated into 4-inch plastic pots. Each pot contained 375 ml of autoclaved Leon

fine sand soil, and were planted to 'Homestead 24' variety of tomato. Following germination the tomato seedlings were thinned to 6 plants per pot. At the end of 21 days and 42 days, respectively, 6 replicates of each treatment were removed. Top and root weights were recorded and all the soil in each pot was processed using Christie and Perry's modified Baermann funnel technique (9). Counts were recorded after 12 hours.

Laboratory Experiment 5. --An investigation into the possibility of a stage resistant to D-D fumigation in the life cycle of Trichodorus christiei.

One pint wide-mouth fruit jars were filled with field soil and each of the following 10 treatments was replicated 5 times:

	Rate per acre	Rate per jar
(1) Check		
(2) 690 gal of mineral spirits		1.0 ml of mineral spirits
(3) 10 gal of D-D per acre		0.2 ml basic solution
(4) 15 " " " "		0.3 " " "
(5) 30 " " " "		0.6 " " "
(6) 50 " " " "		1.0 " " "
(7) 10 gal of water soluble D-D per acre		
(8) 15 " " " " "		
(9) 30 " " " " "		
(10) 50 " " " " "		

The basic solution in treatments 3 through 6 was 92.1 ml mineral spirits + 7.2 ml D-D. The basic solution of the water soluble D-D contained 91.8 ml water + 7.2 ml D-D + 1.0 ml Triton X 100 surfactant. This latter solution was mixed by adding the surfactant to the D-D and then adding water.

The D-D was applied, using a 1 ml pipette filled with a propipette, to a depth of 4 inches within each jar. After the pipette was removed, each injector hole was carefully sealed.

The jars were placed in a laboratory cabinet ($\pm 72^{\circ}\text{F}$) for 2 weeks. Water was added as needed to prevent drying out of the soil. A 100 ml sample of soil was then obtained from each jar and processed using the centrifugal-flotation method. Counts were made of living nematodes; i.e., those which were moving. The remainder of the soil from each jar was placed in a 4-inch clay pot, seeded with 'Homestead 24' variety of tomato and placed in the greenhouse. Three weeks later another 100 ml soil sample was processed as before.

This experiment was repeated using a wider range of water soluble D-D rates and a soil containing a pure population of T. christiei. The treatments included:

	Rate per acre					Rate per jar		
(1) Check								
(2) 25.0 gals of water-soluble D-D per acre						0.33 ml of basic solution		
(3) 45.0 "	"	"	"	"	"	0.60 "	"	"
(4) 56.25 "	"	"	"	"	"	0.75 "	"	"
(5) 75.0 "	"	"	"	"	"	1.00 "	"	"

The basic solution was made up of 88.2 ml water + 10.8 ml D-D + 1.0 ml Triton X 100 surfactant.

Laboratory Experiment 6. --Survival of Trichodorus christiei in the absence of a host at 80°F.

Six clay pots were filled with Leon fine sand in which a pure population of T. christiei had been established, and 4 clay pots with the same type of field soil containing a mixed population of nematodes. The tops of the pots were covered with polyethylene secured by elastic bands, in order to limit the loss of moisture from the soil. The pots were then placed in a constant temperature chamber and watered as needed. Individual pots of each soil type were processed after 0, 2, 4, and 8 weeks. The remaining 2 pots containing the pure populations were taken down after 12 and 16 weeks. Two samples of a 100 ml size were processed from each pot, using the centrifugal-flotation method, and the number of surviving T. christiei were recorded.

Laboratory Experiment 7. --Morphological studies on Trichodorus christiei.

Morphological studies were made on specimens of T. christiei which had been killed and fixed as in Laboratory Experiment 1. However, better results were obtained by staining. The stain was prepared by adding 0.05 ml of green food color (water and propylene glycol) to 2.00 ml of water. Living specimens of T. christiei were placed in this stain which killed them within 24 hours to 48 hours. The killed specimens were then fixed in 1 per cent formalin on Cobb aluminum slides.

RESULTS AND DISCUSSION

Field Experiments

Field Experiment 1. Tables 3 a, b and c show that the build-up of T. christiei following soil fumigation described by Christie and Perry (8) may take place during any season of the year, provided a host is present. However, the more rapid increase obtained on the summer crop of sorghum indicates that the rates of build-up vary with differing levels of temperature.

The depth at which the young feeder roots of the host crop are located is of importance when studying the population of T. christiei (Fig. 1). Tables 3 a, b and c show that the major build-up in the 6 weeks following planting is in the top 6 inches of soil. Thereafter, until harvesting, the population of T. christiei increases very rapidly below the 6-inch depth. The extent to which this deeper population develops is not only dependent upon the nature of the host root system, but also on whether or not the soil has been fumigated (Table 4). The reason for this is apparently due to the fact that soil fumigation permits the young seedlings to develop a root-system capable of outgrowing the ensuing nematode infestation (Fig. 2). Thus, it was possible for the fumigated plots to out-yield those not fumigated (Table 5), while supporting a larger population of T. christiei from about the seventh week until harvesting. However, the control of Belonolaimus longicaudatus by fumigation should not be overlooked since this nema-

tode is far more pathogenic and destructive to vegetable crops in this area than T. christiei is.

When comparing the yield of cabbage from fumigated plots with that from unfumigated plots, it should be noted that there is a significant difference in yield at the 120-day harvest, whereas the yields obtained from the second harvest at 140 days were comparable (Table 5). The crops on fumigated plots of this experiment reached maturity as much as 2 weeks before those on unfumigated plots.

Table 3. Numbers of *Trichodorus christiei* Found in D-D Fumigated Plots Compared to Those in Unfumigated Plots.

Treatment	Sampling Date	Crop Age	Soil Temp. at 6 in.	Sample depth	No. of <i>T. christiei</i> /100 ml of soil	
					Fumigated	Sorghum
(a) Fall Application						
Check	9/30/64 ^a /	0	89° F	0-6 in 6-12 in 0-6 in 6-12 in	25 12 30 14	
Fumigated						
Check	10/31/64	16 days	78°	0-6 in 6-12 in 0-6 in 6-12 in	11b/ 1 0 0	13b/
Fumigated						
Check	11/25/64	42 days	76°	0-6 in 6-12 in 0-6 in 6-12 in	40 5 28 13	85 19 22 8
Fumigated						
Check	12/29/64	76 days	72°	0-6 in 6-12 in 0-6 in 6-12 in	184 147 173 181	191 83 156 110
Fumigated						
Check	1/28/65	106 days	63°	0-6 in 6-12 in 0-6 in 6-12 in	225 203 337 278	166 188 272 244
Fumigated						

^a/ Samples obtained just prior to fumigation.

^b/ Average of 4 replicates.

Harvested 2/3/65.

Table 3. Numbers of Trichodorus christiei Found in D-D Fumigated Plots Compared to Those in Unfumigated Plots.

Treatment	Sampling Date	Crop Age	Soil Temp. at 6 in.	Sample depth	No. of T. christiei/100 ml of soil	
					Cabbage	Sweet corn
Check Fumigated	3/29/65 ^{a/}	4 days	74° F	0-6 in	69	40
				6-12 in	68	55
				0-6 in	3	7
Check Fumigated	4/26/65	32 days	83°	0-6 in	103	219
				6-12 in	105	89
				0-6 in	117	394
Check Fumigated	5/31/65	67 days	85°	0-6 in	84	67
				6-12 in	63	63
				0-6 in	238	273
				6-12 in	375	272

^{a/} Fumigated 18 days prior to this date.

Harvested 6/15/65.

Table 3. Numbers of Trichodorus christiei Found in D-D Fumigated Plots Compared to Those in Unfumigated Plots.

Treatment	Sampling Date	Crop Age	Soil Temp. at 6 in.	Sample depth	<u>No. of T. christiei/100 ml of soil</u>	
					<u>Soybean^{a/}</u>	<u>Sorghum</u>
Check Fumigated	6/30/65 ^{b/}	0	84° F	0-6 in 6-12 in 0-6 in 6-12 in	28 28 0 0	9 11 0 0
	8/2/65	25 days	84°	0-6 in 6-12 in 0-6 in 6-12 in	65 7 11 2	42 7 47 25
Check Fumigated	8/26/65	49 days	86°	0-6 in 6-12 in 0-6 in 6-12 in	42 21 137 56	147 56 312 459
	10/8/65	92 days	82°	0-6 in 6-12 in 0-6 in 6-12 in	39 35 112 137	165 186 221 571

^{a/} Due to poor germination, the soybean plots were replanted 10 days after the initial planting.
^{b/} Fumigated 7 days prior to this date.

Harvested 10/20/65.



Fig. 1. - Roots of a sweet corn plant showing stubby-root symptoms caused by Trichodorus christiei.

Table 4. The Effect of Sorghum Root-depth on Populations of Trichodorus christiei at Harvest.

Depth	Average No. of Root-tips		Dry-weight of roots (grams)		No. of <u>T. christiei</u> /100 ml of soil	
	Check	Fumigated	Check	Fumigated	Check	Fumigated
0-2 in	2 ^a /	3 ^b /	10.1	16.7	182	315
2-4 in	4	6	6.2	6.9	287	350
4-6 in	8	7	2.7	3.8	385	235
6-8 in	4	10	0.9	2.1	228	676
8-10 in	1	4	0.2	0.8	161	392
10-12 in	-	2	0.05	0.4	53	239
12 in	-	1	0.0	0.25	32	189

a/ Average of 6 samples.

b/ Average of 12 samples.

Below a depth of 6 inches, the plant parasitic nematode population became increasingly dominated by T. christiei. In this respect very few B. longicaudatus were found below the 8-inch depth.



Fig. 2. - Comparison of sweet corn seedling growth on unfumigated Leon fine sand soil with that fumigated with D-D soil fumigant applied at 30 gal per acre.

Table 5. Increase in Cabbage Yield and Numbers of Trichodorus christiei Following an Application of D-D Fumigant at 30 Gal per Acre.

Replicate	Yield of cabbage heads			No. of T. christiei/100 ml of soil after 130 days. 0-6 in 6-12 in	
	After 120 Days		After 140 Days		
	No.	Weight (1b)	No.	Weight (1b)	
A	54 ^a /	100.9	34	50.0	441
B	65	142.7	25	39.3	161
C	44	68.4	33	48.4	375
D	34	49.4	40	58.6	252
Average	49.25	90.5 ^b /	33	49.2 ^c /	308
					CHECK
Replicate	D-D			No. of T. christiei/100 ml of soil after 130 days. 0-6 in 6-12 in	
	After 120 Days		No.		
	No.	Weight (1b)	Weight (1b)		
A	77	215.0	18	25.1	
B	73	170.1	29	41.4	
C	53	117.8	26	40.2	
D	54	105.4	37	56.6	
Average	64.25	152.1 ^b /	27.25	40.8 ^c /	

a/ Yield taken from three rows 35 ft long and 2.5 ft wide.

b/ The differences in yields of the D-D plots and Check plots were significant at the 5% level.

c/ Not significantly different.

LSD_{0.05}
(F_{1,3}) = 11.088

Field Experiment 2. When studying the data on the split-plots experiment in Tables 6a, b, c and d, designed to study the residual action of certain nematicides on the build-up of T. christiei, consideration should be given to the following:

- a) The plots treated with the organo-phosphate nematicide, zinophos, restricted the build-up of T. christiei. The residual action of this material also can be seen in the limited numbers of T. christiei which were found on the second crop following a single application (Table 6b). This residual action was not seen in the final crop of sweet corn, though the yields on the plots receiving 2 applications of zinophos proved statistically greater than all other treatments.
- b) The build-up of T. christiei was substantially greater following an application of D-D soil fumigant at 25 gal per acre than at 15 gal per acre. In addition, the numbers of T. christiei present at harvest were greater with a double application of D-D at both the 25 and 15 gal rates.
- c) There were no significant differences in control of T. christiei between single and double applications of Nemagon at 2 gal per acre, and in both cases the build-up surpassed that of the control.
- d) In Table 6a it can be seen that although the plots that received 25 gal of D-D per acre had the highest numbers of T. christiei at harvest, they produced significantly greater yields of corn than did all other treatments. The same was found to be true on cabbages (Tables 6b and c) with the exception that the yield was not statistically different from those plots receiving 2 applications of zinophos at 3 lb per acre.

e) Table 6a shows that the application of each nematicide resulted in significant yield increases of field corn. These yield increases were partly due to early control of T. christiei and partly due to control of other parasitic nematodes.

In addition, the number of T. christiei after 58 days was greater than that at 72 days. The reason for this is apparently due to the fact that at harvest the samples were taken to a 6-inch depth. As shown in the previous experiment, by crop maturity the majority of T. christiei are below this depth.

When cabbage was planted on the same plots following the corn and half the original number of plots retreated, none produced significant yield increases except those retreated with zinophos at 3 lb per acre and D-D at 25 gal per acre (Table 6c). The fact that the check plots yielded more than some of the treated plots suggests that cabbage, by virtue of their numerous rootlets, can withstand nematode damage better than corn. In addition, the larger initial populations of T. christiei may have influenced yields.

f) The counts of T. christiei on the final untreated crop of sweet corn are given in Table 6d. With the exception of Nemagon, the counts of all treatments are very similar at harvest, although the numbers 3 weeks after planting show considerable variation. The only statistically outstanding yield was obtained from the plots which had received 2 applications of zinophos at 3 lb per acre.

Table 6a. A Comparison of the Effects Certain Nematicides Have On the Build-up of Trichodorus christiei on 'Funks' Hybrid Field Corn.

Plot No.	Treatment	Numbers of <u>T. christiei</u> per 100 ml. of soil		Weight of 60 corn plants
		After 58 days	After 72 days (Harvest)	
1 + 2	Check	101 ^a /	172	15.60 1b
3 + 4	Zinophos 3 lb./acre	11	54	34.82 1b
5 + 6	Nemagon 2 gal/acre	393	392	29.44 1b
7 + 8	D-D 15 gal/acre	721	391	34.57 1b
9 + 10	D-D 25 gal/acre	833	462	43.38 1b

^a/ Average of 8 replicates.
— Yield data $F_{27}^2 = 12.34**$; i.e., significant at the 1% level.

Duncan Multiple Range Test on yield at the 5% level

Plot No.	2	1	5	6	7	3	8	4	10	9
13.833	17.375	27.438	31.438	33.250	33.625	35.875	36.000		41.563	45.188

Table 6b. A Comparison of the Residual Effects of a Split Application of Certain Nematicides on the Build-up of Trichodorus christiei on Cabbage.

Plot No.	Treatment	No. Applications ^a /	Numbers of <u>T. christiei</u> in 100 ml of soil at various intervals after planting.				
			At Fumigation	21 Days	50 Days	80 Days	112 Days(Harvest)
1	Check		28	25	74	49	193
2	Check		33	28	63	105	217
3	Zinophos	2	28	8	11	11	25
4	3 lb/acre	1	35	28	39	60	119
5	Nemagon	2	70	84	63	84	249
6	2 gal/acre	1	117	105	95	95	280
7	D-D	2	100	35	172	168	441
8	15 gal/acre	1	220	46	60	-	235
9	D-D	2	238	14	98	154	529
10	25 gal/acre	1	247	105	46	60	371

^a/ 1st Application -- made prior to the previous crop of corn.

2nd Application -- made prior to planting the cabbage.

Table 6c. Effect of Nematicide Treatments on Total Number and Weight of Cabbage Heads on Three Harvest Dates.

Plot	Treatment	No. Applications	Harvest Date				Total Harvest			
			1/27/66		2/18/66		2/25/66		No.	Wt. (lb)
1	Check		15	21.2	75	83.9	39	36.7	129	141.8
2	Check		20	29.5	83	84.4	35	27.8	138	141.7
3	Zinophos	2	47	58.7	101	116.6	39	33.9	187	209.2
4	3 lb/acre	1	18	27.3	77	79.1	42	39.5	137	145.9
5	Nemagon	2	10	12.6	57	64.6	54	48.5	121	125.7
6	2 gal/acre	1	11	13.0	64	72.6	39	38.9	114	124.5
7	D-D	2	26	39.2	63	71.8	50	45.5	139	156.5
8	15 gal/acre	1	15	21.3	59	61.4	51	45.4	125	128.1
9	D-D	2	58	85.9	78	91.0	50	47.4	186	224.3
10	25 gal/acre	1	30	38.0	79	86.7	29	23.5	138	148.2

Yield data $F_{27}^9 = 2.3096^*$; i.e., significant at the 5% level.

Plot No.	6	5	Duncan Multiple Range Test on yield at the 5% level.
31.125	31.425	32.025	35.425 35.525 36.475 37.050 39.125 32.3 56.075

Table 6d. A Comparison of the Residual Effects Certain Nematicides Have on the Build-up of Trichodorus christiei On Unfumigated 'Gold Cup' Sweet Corn.

Plot No.	Previous Treatment	No. Applications	No. of <u>T. christiei</u> per 100 ml. of soil.		Yield of Corn Ears Wt. (lb.)
			After 24 Days	After 84 Days	
1	Check		112 ^{a/}	126	33.8
2	Check		88	74	40.5
3	Zinophos	2	14	123	49.1
4	3 lb/acre	1	63	133	34.5
5	Nemagon	2	200	210	39.3
6	2 gal/acre	1	119	179	35.0
7	D-D	2	112	109	34.8
8	1.5 gal/acre	1	42	119	40.0
9	D-D	2	105	133	40.9
10	2.5 gal/acre	1	53	140	40.3

a/ Average of 4 replicates.
Yield data $F_{27}^9 = 2.77^*$; i.e., significant at the 5% level.

Duncan Multiple Range Test on yield at the 5% level.

Plot No.	1	4	7	5	8	10	2	9	3
33.8	34.5	34.8	35.0	39.3	40.0	40.3	40.5	40.9	49.1

Field Experiment 3. Table 7 shows that in the absence of a host there was a gradual mortality of T. christiei during the first 8 weeks. Between the eighth and twelfth weeks the mortality doubled that of the first 8 weeks. After 16 weeks, when the experiment was terminated, the T. christiei population had been reduced to 1/5 of the original number. A comparison of the survival of T. christiei under bare plots with bare plots in which organic matter had been incorporated (at the rate of 17 tons per acre) revealed no differences (Table 7). By contrast, Patrick et al. (23) obtained nematicidal substances from decomposing rye. It is possible that under summer conditions a more rapid decomposition of the organic matter might prove detrimental to T. christiei.

Table 7. A Comparison of the Survival of Trichodorus christiei in the Presence and Absence of Organic Matter (O.M.) Under Clean Cultivation in the Field.

Treatment	Time in Weeks									
	0 Check	0.M.	3 1/2 Check	0.M.	8 Check	0.M.	12 Check	0.M.	16 Check	0.M.
No. of <u>T. christiei</u> / 100 ml of soil.	414 ^a /	390	340	350	296	288	120	133	94	74
% reduction from original population.	0	0	18	10	29	26	71	71	77	81
Soil Temp. (°F)	74								61	

^a/ Data from 4 replicates.

Greenhouse Experiments

Greenhouse Experiment 1. Of the four temperatures investigated, 80° F proved most favorable for reproduction by T. christiei (Table 8). There was, however, no statistical significance between the reproductive rates at 80° and 85° F. Malek et al. (20) in New Jersey found 77° F to be the optimum for T. christiei reproduction. Thus, the New Jersey and Florida populations of T. christiei appear to have similar optimum temperature requirements for reproduction.

Table 8. The Effect of Temperature on the Reproductive Rate of 25 Trichodorus christiei on Sweet Corn and Tomato After 8 Weeks.

Temperature	Rep. 1 ^a /		Rep. 2		Average	
	Corn	Tomato	Corn	Tomato	Corn	Tomato
95°F	5 ^b /	53 ^b /	583	2,844	294	1,449
90°F	2,253	10,500	5,103	7,489	3,678	8,995
85°F	8,394	13,828	6,899	11,567	7,647	12,698
80°F	9,891	15,402	10,626	12,788	10,259	14,095

^a/ Replicate 1 ran from 8/1/65 to 9/25/65; and Replicate 2, from 11/5/65 to 1/2/66.

^b/ Average count of 8 pots.

$$\text{Corn } F_3^3 = 24.40^*$$

LSD

05 3,991.34

$$\text{Tomato } F_3^3 = 63.2^{**}$$

LSD

05 2,591.45

The populations of T. christiei produced on 'Rutgers' tomato were greater in number than those produced on 'Gold Cup' sweet corn. As noted by Bird (4), the morphology of T. christiei was affected by the host upon which the specimens had been feeding. Of interest was the

presence of several males at 95°F. Under field conditions males of this nematode are extremely rare.

Greenhouse Experiment 2. The data presented in Table 9 show that T. christiei and B. longicaudatus each parasitize and reproduce on pangola-grass, giving rise to as much as an eightfold increase in their numbers over a three-month period in sterile soil. In unsterilized soil taken from two different field locations, the populations did little more than maintain their numbers, which suggested the presence of a biological interaction. Consideration should be given to two factors in unsterilized soil, viz., (1) the presence of other plant parasitic nematodes and (2) the presence of certain nematode predators.

T. christiei proved more pathogenic to the pangolagrass root system than did B. longicaudatus (Table 10), but the latter was more pathogenic to sweet corn. The mixed population of plant parasitic nematodes present in "Pangola" and "Crop" soils undoubtedly contributed to the smaller root systems in these soils when compared to those in "Sterile" soil. Albeit, the terminal numbers of T. christiei and B. longicaudatus were larger in the "Sterile" soil.

Table 9. Plant Parasitic Nematode Populations in 100 ml of Soil From Under Pangolagrass Followed by Sweet Corn and Vice Versa Maintained in the Greenhouse for 3 Months.

Soil	Host	Initially inoculated with 25 <i>T. christiei</i>				
		Stubby-root	Lesion	Stunt	Sting	Ring
"Sterile"	Pangolagrass then Sweet Corn	14 ^a / 907	- -	- -	- -	- -
"Pangola"	Pangolagrass then Sweet Corn	11 151	39 5	- -	- -	- -
"Crop"	Pangolagrass then Sweet Corn	14 109	42 1	2 1	- -	83 -
"Sterile"	Sweet Corn then Pangolagrass	54 459	- -	- -	- -	- -
"Pangola"	Sweet Corn then Pangolagrass	94 119	188 32	- -	- -	- -
"Crop"	Sweet Corn then Pangolagrass	88 105	130 25	219 70	- -	5 -

Soil	Host	Initially inoculated with 25 <i>Belonolaimus</i> <i>longicaudatus</i>				
		Stubby-root	Lesion	Stunt	Sting	Ring
"Sterile"	Pangolagrass then Sweet Corn	- -	- -	- -	53 315	- -
"Pangola"	Pangolagrass then Sweet Corn	- 21	46 25	- -	7 11	4 -
"Crop"	Pangolagrass then Sweet Corn	- -	27 4	1 -	7 -	130 67
"Sterile"	Sweet Corn then Pangolagrass	- -	- -	- -	112 872	- -
"Pangola"	Sweet Corn then Pangolagrass	28 60	147 49	- -	32 42	- -
"Crop"	Sweet Corn then Pangolagrass	16 112	210 25	172 144	18 6	58 14

^a/ Average of three samples. Samples collected by taking one probe from each of the five 6-inch pot replicates. A 100 ml sub-sample was processed from each sample.

Key to Table 9:

Stubby-root	=	<u>Trichodorus christiei</u>
Lesion	=	<u>Pratylenchus brachyurus</u> and <u>P. zae</u>
Stunt	=	<u>Tylenchorhynchus</u> spp.
Sting	=	<u>Belonolaimus longicaudatus</u>
Ring	=	<u>Criconemoides</u> spp.

Table 10. Total Dry Weight (Grams) of Roots From 5 Replicates of Pangolagrass and Sweet Corn After 3 Months in Association with Trichodorus christiei and Belonolaimus longicaudatus.

Soil	Crop	Nematode			
		<u>Stubby-root</u> on 11/29/65	<u>Stubby-root</u> on 3/7/66	<u>Sting</u> on 11/29/65	<u>Sting</u> on 3/7/66
"Sterile"	Sweet Corn	32.2 ^a /	21.4 ^b /	17.5 ^a /	18.7 ^b /
	Pangolagrass	14.8 ^b /	14.9 ^a /	25.8 ^b /	18.2 ^a /
"Pangola"	Sweet Corn	16.6	18.9	9.5	17.9
	Pangolagrass	6.6	10.5	9.9	12.3
"Crop"	Sweet Corn	17.1	16.2	12.2	15.1
	Pangolagrass	12.6	9.6	10.7	18.1

^a/ Same pots; planted first to sweet corn, then pangolagrass.

^b/ Same pots; planted first to pangolagrass, then sweet corn.

Greenhouse Experiment 3. The results of the experiment conducted to determine the effect of the competition by B. longicaudatus on the reproduction of T. christiei are presented in Table 11. After 45 days virtually no discernible effects of the competition were detected. In all probability, this was due to the low levels of initial inoculum. The data recorded 45 days after replanting these same pots suggest that T. christiei in the control pots had attained maximum levels of infestation; i.e., \pm 1,000 individuals per 100 ml of soil, irrespective of the initial level of inoculum. Nevertheless, the presence of B. longicaudatus resulted in a marked reduction of the numbers of T. christiei. This reduction was more marked the greater the initial inoculum numbers, averaging up to 50 per cent. These differences were not statistically significant, owing to wide variation in numbers and too few replicates. By contrast, the reproduction of B. longicaudatus was less effected the larger the initial numbers of T. christiei.

The average dry weights of the second sweet corn planting; i.e., from the 45th day to the 90th day, are given in Table 12. These data indicate that B. longicaudatus is much more pathogenic to sweet corn than is T. christiei (Fig. 3 and 4). In general the corn was damaged more by the combination of the species than by B. longicaudatus alone, though there were fewer numbers of B. longicaudatus in the combination.

Table 11.

The Interrelationship of Two Ectoparasitic Nematodes At Different Rates
of Initial Inoculum While Feeding on Sweet Corn in 6-inch Plastic Pots.

Nematode	Initial Rate of Inoculum	AFTER 45 DAYS IN 100 ML OF SOIL				Average
		1	2	3	4	
AFTER 90 DAYS IN 100 ML OF SOIL						
Stubby-root ^{a/}	25	18	53	60	95	57
Sting ^{b/}	25	28	35	39	38	35
Stubby-root+Sting	25+25	39+25	21+11	60+70	63+28	46+34
Stubby-root	50	46	63	60	77	60
Sting	50	91	119	53	67	83
Stubby-root+Sting	50+50	53+56	46+21	84+116	133+140	79+83
Stubby-root	100	112	112	112	196	133
Sting	100	105	196	179	196	169
Stubby-root+Sting	100+100	81+133	49+196	60+119	158+242	87+173
Stubby-root	250	312	207	224	210	238
Sting	250	375	469	413	256	378
Stubby-root+Sting	250+250	281+392	186+161	382+291	130+102	246+239
AFTER 90 DAYS IN 100 ML OF SOIL						
Sticky-root	25	1,106	1,407	525	1,162	1,050
Sting	25	315	574	578	217	421
Stubby-root+Sting	25+25	412+210	1,421+322	518+392	480+144	708+267
Stubby-root	50	1,582	1,001	896	987	1,116.5
Sting	50	651	501	602	378	533
Sticky-root+Sting	50+50	721+396	805+329	284+305	991+284	700+328.5
Stubby-root	100	1,897	665	1,194	770	1,131.5
Sting	100	546	1,117	1,001	1,099	941
Sticky-root+Sting	100+100	966+574	546+917	140+718	536+473	547+670.5
Stubby-root	250	1,512	1,456	399	599	991.5
Sting	250	991	438	564	1,236	807
Sticky-root+Sting	250+250	308+448	585+756	273+819	851+515	504+634.5

^{a/} Stubby-root = *Trichodorus christiei*.
^{b/} Sting = *Belonolaimus longicaudatus*.

Table 12. Effect of Trichodorus christiei and Belonolaimus longicaudatus on the Dry Weights (Grams) of the Second Crop of Sweet Corn. The Corn Was Harvested 45 Days after Replanting in Pots in Which the Nematodes had Previously Been Established.

Initial Inoculum		<u>T. christiei</u>	<u>B. longicaudatus</u>	<u>T. christiei</u> + <u>B. longicaudatus</u>
25	Tops	4.0 ^{a/}	3.6	3.4
	Roots	2.9	2.3	2.0
50	Tops	3.9	3.5	3.7
	Roots	3.2	2.7	2.3
100	Tops	3.4	2.8	3.4
	Roots	2.7	2.2	1.9
250	Tops	4.0	1.7	2.5
	Roots	2.3	1.2	1.6
Check	Tops	4.1		
	Roots	3.9		

a/ Average of 4 replicates with three plants per replicate.



Fig. 3 - The effect of Belonolaimus longicaudatus on the growth of sweet corn 45 days after replanting in pots initially inoculated with (left to right) 250, 100, 50 and 25 specimens.



Fig. 4 - Growth of sweet corn 45 days after replanting in pots initially inoculated with (left to right) 250, 100, 50 and 25 Trichodorus christiei.

Greenhouse Experiment 4. Results of the comparison of the reproductive rates of T. christiei in D-D fumigated soil with unfumigated soil are given in Table 13. After 8 weeks the number in the unfumigated soil was 1.7 times that in fumigated soil, due to higher initial numbers of T. christiei in the unfumigated soil. However, during the following 8 weeks the situation was reversed with D-D fumigated soil having 1.6 times more T. christiei. This demonstrated that a build-up of T. christiei, similar to that seen in the field, can take place when this nematode is inoculated into D-D fumigated soil in greenhouse pots.

Table 13. The Reproductive Rate of Trichodorus christiei in D-D Fumigated Soil and Unfumigated Soil in Greenhouse Pots.

Treatment	Numbers of <u>T. christiei</u> in 100 ml of soil		
	Initially	After 8 weeks	After 16 weeks
Unfumigated	10	Host: Oats 72 ^a /	Host: Sweet Corn 560
D-D at 30 gal per acre	2	42	910

^a/ Average of 2 samples which consisted of a probe taken from each of the 6 replicates.

Greenhouse Experiment 5. The data obtained in the experiment conducted to determine whether oligochaetes have an effect on the reproduction of T. christiei are presented in Table 14. It should be noted that, although initially inoculated into steam-sterilized soil, the oligochaetes were able to feed and reproduce. However, no statistical difference

could be detected on the reproduction of T. christiei, under the conditions of the experiment, as a result of the increase in oligochaetes.

Table 14. Effects of Oligochaetes on Number Of
Trichodorus christiei After 8 Weeks.

Inoculum	No. <u>T. christiei</u> per 100 ml of soil	No. Oligochaetes per 100 ml of soil
100 <u>T. christiei</u> alone	1,419 ^a /	--
100 <u>T. christiei</u> + 100 oligo-chaetes	1,326	806
100 <u>T. christiei</u> + 500 oligo-chaetes	1,285	1,238

^a/ Average of 5 replicates.

The results of the study conducted on the possible predaceous habits of Eudorylaimus simplex, Aporcelaimellus obscurus, and Mylonchulus parabrechyurus on T. christiei are shown in Table 15. Of these three free-living nematodes, only the presence of A. obscurus resulted in reduced numbers of T. christiei. However, in a second test when specimens of A. obscurus were inoculated into a previously established population of T. christiei, no such differences were observed (Table 16). From these tests it was concluded that none of these nematodes affect population levels of T. christiei.

Table 15. Effects of Three Possible Predaceous Nematodes on Populations of Trichodorus christiei.

	Numbers per 100 ml of soil after:	
	8 Weeks	25 Weeks
100 <u>T. christiei</u> alone	270	147
100 <u>T. christiei</u> + 25 <u>Eudorylaimus simplex</u>	266 0	228 84
100 <u>T. christiei</u> + 100 <u>Eudorylaimus simplex</u>	238 4	144 133
25 <u>Eudorylaimus simplex</u> alone	4	109
	Numbers per 100 ml of soil after:	
	8 Weeks	16 Weeks
100 <u>T. christiei</u> alone	203	591
100 <u>T. christiei</u> + 25 <u>Aporcelaimellus obscurus</u>	217 0	353 66
100 <u>T. christiei</u> + 100 <u>Aporcelaimellus obscurus</u>	154 4	273 96
	Numbers per 100 ml of soil after:	
	8 Weeks	25 Weeks
100 <u>T. christiei</u> alone	270	147
100 <u>T. christiei</u> + 25 <u>Mylonchulus parabrachyurus</u>	207 0	196 91
100 <u>T. christiei</u> + 100 <u>Mylonchulus parabrachyurus</u>	221 4	210 203
25 <u>Mylonchulus parabrachyurus</u> alone	0	420

Table 16. Effect of Aporcelaimellus obscurus on Established Populations of Trichodorus christiei.

Inoculum	Numbers per 100 ml of soil after 12 weeks:			
	1	2	3	Average
<u>T. christiei</u> alone	805	630	735	723
<u>T. christiei</u> + 100 <u>A. obscurus</u>	833 315	578 305	704 291	705 304

Greenhouse Experiment 6. Results of the experiment conducted to determine which of four potting containers provided the most ideal environment for the reproduction of T. christiei are given in Table 17. In the greenhouse it was found that the glazed crock was statistically superior to plastic, to sub-surface irrigated clay, and to surface irrigated clay pots; there being no statistically significant difference among these latter three. In the constant temperature chamber, however, the plastic container was superior. In this case there was no statistically significant difference between the glazed crock and two types of clay pots. Similar data were obtained in a repetition of the constant temperature experiment in a different kind of chamber. Figure 5 shows the root system of sweet corn in three of the containers. Note the preponderance of roots lining the walls of the clay pot.

These data suggest that, in conditions where the greatest variable is temperature, glazed crocks are desirable, while in those conditions in which moisture is the most important variable, the plastic pots are best suited for rearing T. christiei.

Table 17. Numbers of T. christiei in 100 ml of Soil from Four Different Containers Using Sweet Corn as a Host After a Period of 8 Weeks.

Container	Numbers of <u>T. christiei</u> per 100 ml of soil		Average dry weight of roots per pot	
	Greenhouse	Chamber	Greenhouse	Chamber
Plastic Pot	939 ^a /	1,209 ^b /	1.7	2.6
Glazed Crock	1,303	751	2.0	2.4
Clay Pot(surface irrigated)	608	783	3.5	1.9
Clay Pot(sub-surface irrigated)	885	796	1.7	3.5

^a/ Two plants per pot.

^b/ Four plants per pot.



Fig. 5. - Root growth of sweet corn after 8 weeks in the greenhouse in a clay pot, glazed crock, and plastic pot.

Duncan's Multiple Range Test:

Greenhouse =	Surface	Sub-Surface	<u>Plastic</u>	<u>Glazed</u>
	Irrigated <u>Clay</u>	Irrigated <u>Clay</u>		
	608	885	939	<u>1,303</u>
Growth chamber =	Glazed	Surface	Sub-Surface	Plastic
		Irrigated <u>Clay</u>	Irrigated <u>Clay</u>	
	751	786	796	<u>1,209</u>

Laboratory Experiments

Laboratory Experiment 1. The results of a study on the life cycle of *T. christiei* following inoculation of 10 gravid females onto tomato seedlings growing at 80°F are given in Table 18. The different stages in the life cycle (Fig. 6) were first recorded on the following days after inoculation:

1st Larval stage	4 days
2nd Larval stage	4-5 days
3rd Larval stage	7 days
4th Larval stage	10 days
Adult.	14 days
Gravid female	17-18 days

The criterion used to determine the stage of development was the size of the gonadal primordium. The reason for this can be seen in Fig. 7, which shows the size variation in adult *T. christiei* from two different habitats. Measurements of 10 specimens were made at molting in 1 per cent formalin. The average sizes were:

Table 18. Study of the Life Cycle of the Offspring of 10 Gravid Female Trichodorus christiei at 80°F While Feeding on 'Homestead 24' Tomato.

No. Days After Inoculation	Test	Original Adults Surviving	Life Cycle Stage					Av. No. of Offspring
			Adult	4th	3rd	2nd	1st	
3	1	3.0						0
	2	3.0						
4	1	7.3					1.5	1.5
	2	7.0					1.0	1.0
5	1	3.8				3.8	3.3	7.1
	2	7.5					3.0	3.0
6	1	5.0				9.5	1.8	11.3
	2	4.5				1.5	2.5	4.0
7	1	6.8			1.5	12.3	2.3	16.1
	2	7.5			1.0	5.5	-	6.5
8	1	5.5			7.8	16.5	4.3	28.6
	2	4.5			7.0	21.5	-	28.5
9	1	7.5			8.8	13.8	1.8	24.4
	2	2.0			3.5	7.0	-	10.5
10	1	3.3		3.0	15.0	22.0	3.0	43.0
	2	3.5		-	22.5	13.0	-	35.5
11	1	8.0		7.3	17.5	18.5	4.8	48.1
	2	5.5		10.5	8.5	15.5	0.5	35.0
12	1	4.0		17.3	26.8	14.5	2.8	61.4
	2	3.5		21.0	10.0	16.5	2.0	49.5
13	1	4.5		14.8	21.8	14.5	3.3	54.4
	2	4.5		19.5	5.5	8.0	0.5	33.5
14	1	5.5		16.0	15.8	7.5	1.0	40.3
	2	5.5		44.0	25.5	6.0	0.5	76.0
15	1	3.0	2.3	13.5	9.5	10.0	2.3	37.6
	2	7.0	7.5	50.0	22.0	9.0	0	88.5
16	1	3.5	10.3	15.8	17.3	7.5	1.0	51.9
	2	3.0	11.0	12.5	6.5	2.0	0	32.0
17	1	3.8	16.5	27.3	22.0	13.0	2.5	81.3
	2	0.5	5.5 ^{a/}	15.5	11.5	5.0	1.5	39.0
18	1	3.0	47.5 ^{a/}	28.3	27.5	18.8	2.8	125.0

^{a/} Gravid females recovered.

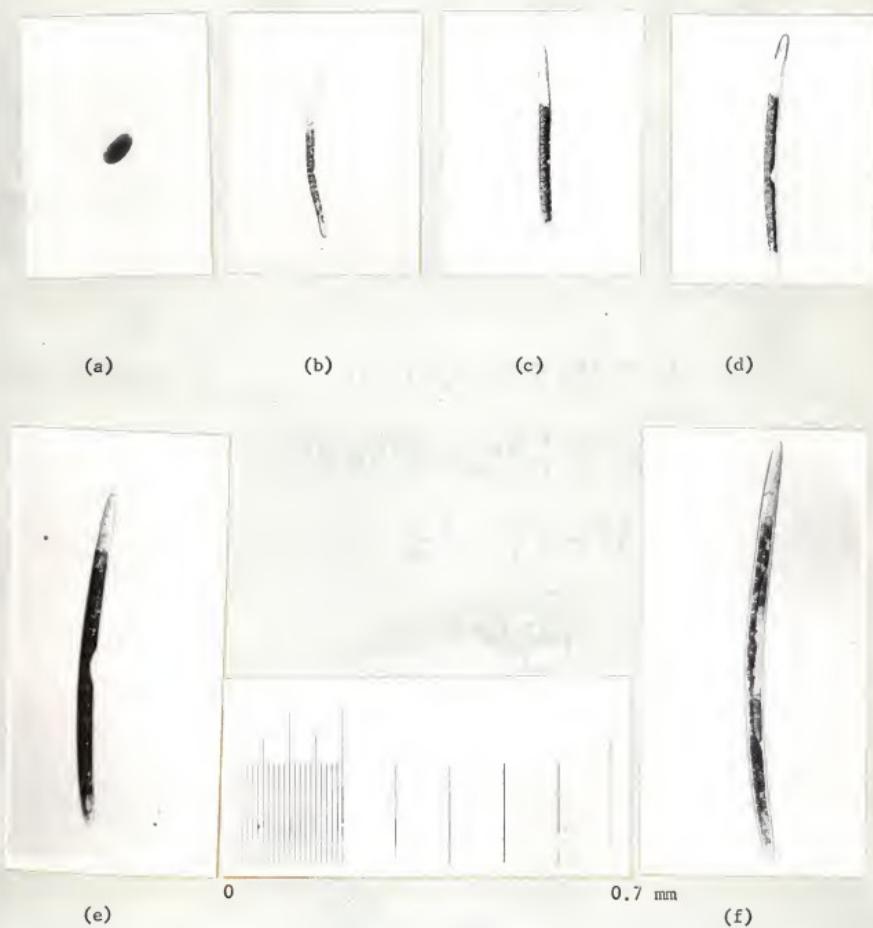


Fig. 6 - Stages in the life cycle of *Trichodorus christiei* at (10X);
(a) egg, (b) first larval stage, (c) second larval stage;
(d) third larval stage, (e) fourth larval stage, and (f)
adult female.



Fig. 7 - Variation in size between adults of Trichodorus christiei collected from two locations near Sanford, Florida.

	<u>Length</u>	<u>a</u>	<u>b</u>	<u>c</u>	<u>Gonad Length</u>
1st-2nd	0.311mm	20.7	2.9	124.3	.008mm
2nd-3rd	0.41mm	16.2	3.7	98.3	0.03mm
3rd-4th	0.51mm	16.3	4.3	126.6	0.04mm
4th-Adult	0.70mm	16.0	5.6	100.0	0.23mm

Laboratory Experiment 2. The molting of T. christiei proved different than that of any other plant parasitic nematode. Prior to molting the specimens of T. christiei became quiescent. The posterior intestinal contents were ejected, while the remainder became granular with the denser material collecting around the perimeter of the intestine. This collection was most pronounced ventrally. Quiescence was terminated by a movement of the neck region from side to side, with the inner cuticle of the neck region becoming markedly wrinkled on the side to which the head was tilted. Concurrently the cuticle was, at times, stretched over the head as seen in Fig. 12. During these movements, esophageal palpitations were observed and the gland nuclei were clearly defined.

The old cuticle loosened, giving rise to a small overlap at the head and tail. This was followed by a break in the old cuticle circumventing the body in the region of the base of the onchiostyle (Fig. 8). By contraction on the head a "hood" was formed on the dorsum which usually remained attached to the old cuticle at a single point (Fig. 9). This formation of a hood was somewhat similar to that recorded by Lapage (19) for infective larvae of Trichostrongylus, Haemonchus and Ostertagia.



Fig. 8 - Break in the old cuticle prior to molting of its "hood" by Trichodorus christiei.



Fig. 9 - A molting specimen of Trichodorus christiei.
Note the absence of the onchiostyle in the
"hood."

The head of T. christiei was then withdrawn from the hood.

Note in Fig. 9 that the portion of the body which had emerged from the old cuticle was greater in diameter than the portion still contained within both cuticles. The old cuticle was then shed within a few minutes by movements of the animal.

Detailed examinations of the head revealed the amphidial linings to be clearly visible, but no portion of the onchiostyle was found in any of the four molted cuticles. The tripartite onchiostyle of Trichodorus is unique among the plant parasitic nematodes as is the molting process.

Laboratory Experiment 3. The reproductive rates of individual specimens of T. christiei are given in Table 19. A marked increase (76%) in the numbers of offspring recovered occurred between the 16th and 17th days. Thereafter, until the 19th day, the increase was gradual. Between the 19th and 38th days, the population increased 12.2 times. Thus, although as many as 30 offspring were recovered from a single specimen in one generation, the average reproductive rate of this nematode is 12 offspring per 19 days at 80°F using 'Homestead 24' tomato as the host plant. This figure was further substantiated by the data in Table 20a, prior to the influence of population pressures.

Laboratory Experiment 4. A study was made on the effect of varying initial inoculum numbers on the reproductive rate of T. christiei. The data from this study, using 6 'Homestead 24' tomato seedlings per 4-inch plastic pot of sterile soil, are presented in Table 20a. After

Table 19. Reproductive Rate of Individual Specimens of *L. christiei* While Feeding on 'Homestead 24' Tomato Seedlings Growing at a Constant Temperature of 80° F.

Days after inoculation	Test number	Surviving original individuals divided by no. pots with Offspring		Numbers of the various stages in the life cycle recovered; mean of pots with offspring			Daily ^a / 4th	Daily ^a / 3rd	Total Max.	Daily Avg.
		Adult	4th	2nd	1st					
16	1	5/5	--	2.2	2.6	0.8b/	--	5.6	11	5.9
	2	2/6	--	0.7	2.8	2.0	0.7	6.2	12	
17	1	4/4	0.9	3.2	4.9	3.9	--	12.8	27	10.4
	2	1/5	0.4	1.8	3.4	2.0	0.4	8.0	16	
18	1	5/3	0.6	4.0	5.0	2.8	--	12.4	29	11.0
	2	2/6	0.2	3.2	4.5	1.8	0	9.7	17	
19	1	4/6	2.7	2.8	2.8	2.0	--	12.4	21	11.6
	2	1/6	1.7	4.3	3.7	2.3	0.8	12.8	30	
38	1						163	352	141	
	2						119	219		

a/ Represents the maximum number of offspring from a single individual.

b/ In the first test the figure given in the 2nd larval stage column represents the combined total of 1st and 2nd larval stages.

Table 20a. Reproductive Rates of *Trichodorus christiei*
at Different Rates of Infestation Maintained
at 80°F.

Inoculum level	Number of <i>T. christiei</i> per 4-inch pot (400 ml)			
	At 21 Days	Increase per individual	At 42 Days	Increase per individual
25	274 ^a /	10.9	4,092 ^b /	163.7
100	1,253	12.5	5,786	57.8
400	1,823	4.6	7,030	17.6

^a/ Average of 6 replicates.

^b/ Average of 12 replicates.

Table 20b. Average Weights of 'Homestead 24' Tomatoes
Inoculated with *Trichodorus christiei*.

	Fresh Weight (grams)		Dry Weight (grams)	
	After 21 days	After 42 days	After 21 days	After 42 days
25 Tops	3.2 ^a /	8.1 ^b /	0.167	0.675
Roots	0.7	1.60	0.062	0.213
100 Tops	3.5	7.8	0.200	0.658
Roots	0.8	1.55	0.073	0.224
400 Tops	2.7	6.3	0.150	0.450
Roots	0.7	1.45	0.055	0.199

^a/ Average of 6 pots; 6 plants per pot.

^b/ Average of 12 pots; 6 plants per pot.

21 days the greatest increases were found in those pots initially inoculated with 100 specimens. This was closely followed by those with 25 specimens. After 42 days the pots originally having 25 individuals produced increases of almost three times the rate of those with 100 and over nine times the rate of those with 400, thus demonstrating the importance of population pressures on the reproductive rate of T. christiei. Alhassan and Hollis (1) attributed the slower rise of higher initial inoculum numbers of T. christiei to an interaction with the host. Since there was an absence of necrosis, these authors have termed this relation one of "balanced parasitism." Figs. 10 and 11 show T. christiei feeding on tomato roots in agar.

Data relating to seedling weights are given in Table 20b. No statistical difference was found between the fresh weights of the tops or the roots when the treatments were analyzed after 21 days and 42 days, respectively. Alhassan and Hollis (1) obtained similar results on cotton seedlings analyzed 21 days after inoculation with 0, 100, and 400 specimens of T. christiei. Nevertheless, their conclusion was that "seedling weights were related inversely to both initial and final populations of the nematode." The same conclusion may be drawn from the above experiment on 'Homestead 24' tomatoes after 42 days.

Laboratory Experiment 5. Results from the investigation into the possibility of resistance in the eggs of T. christiei to D-D fumigation are given in Tables 21a and b. Under the conditions of the experiment; i.e., recording the nematode population 2 weeks after fumigation, and 3 weeks after planting a susceptible host, it was found that whenever



Fig. 10 - Characteristic position of *Trichodorus christiei* while feeding; i.e., the head at right angles to the cell upon which it is feeding.



Fig. 11 - *Trichodorus christiei* feeding upon the root-cap of tomato in agar.

Table 21a. Effects of D-D Fumigation on Trichodorus christiei,
Belenolaimus longicaudatus and Hoplolaimus galeatus,
in Soil Contained in Pint Jars.

Treatment	Average numbers ^a / of nematodes per 100 ml of soil 2 weeks after fumigation (column 1), and 3 weeks after planting tomato (column 2)			
	<u>Trichodorus christiei</u>		<u>Belenolaimus longicaudatus</u>	
	1	2	1	2
1) Check	349	253	70	70
2) Mineral spirits 690 gal/acre	120	111	69	41
3) D-D 10 gal/acre	198	141	2	10
4) D-D 15 gal/acre	156	109	1	3
5) D-D 30 gal/acre	35	32	0	0
6) D-D 50 gal/acre	11	24	0	3
7) Emulsified D-D 10 gal/acre	231	143	14	8
8) Emulsified D-D 15 gal/acre	160	152	5	1
9) Emulsified D-D 30 gal/acre	60	117	0	1
10) Emulsified D-D 50 gal/acre	16	59	0	0

a/ Average of 5 replicates.

Table 21b.
Effects of D-D Fumigation on a Pure Population
of Trichodorus christiei in Soil Contained in
Pint Jars.

Treatment	Average numbers ^a of <u>T. christiei</u> per 100 ml of soil 2 weeks after fumigation, and 3 weeks after planting.
1) Check	354 420
2) D-D Emulsified 25 gal/acre	51 167
3) D-D Emulsified 45 gal/acre	23 37
4) D-D Emulsified 56.25 gal/acre	0 0
5) D-D Emulsified 75 gal/acre	0 0

^a/ Average of 4 replicates.

the adults and larvae were killed no progeny of T. christiei could be found. This indicated that the eggs were killed.

Adults and larvae were found to survive following applications of D-D at 50 gal per acre to the jars, whether applied in mineral spirits or in a water-emulsified state. However, it would appear that many of these surviving specimens were incapable of infecting susceptible hosts. This phenomenon appeared more pronounced at the lower application rates of D-D.

The data in Table 21a indicate that T. christiei is more tolerant to D-D fumigation than is B. longicaudatus or Hoplolaimus galeatus. This experiment also demonstrates the marked susceptibility of B. longicaudatus to D-D fumigation.

Laboratory Experiment 6. The survival of T. christiei in the absence of a host was studied at a constant temperature of 80°F (Table 22). The rate of mortality of T. christiei was slightly greater in field soil than that of a pure population which previously had been established in sterile soil. This difference, however, was considered insufficient to indicate the presence of a successful predator.

A comparison of the data obtained for survival at 80°F (Table 22) with those obtained in the field (Table 8) shows the rates of mortality at 80°F to be slightly greater during the first 8 weeks. Thereafter, the field population declined much more rapidly until at the 16th week there was a difference of 20 per cent.

Often fallowing is associated with dry tillage. After allowing 6-inch clay pots of soil containing T. christiei to dry out in an air-conditioned laboratory to a moisture level as low as 1.7 per cent,

it was found that live specimens could still be recovered. Specimens of T. christiei recovered from this soil characteristically possessed a thickened cuticle (Fig. 12). This cuticular thickening suggests that

Table 22. Survival of Trichodorus christiei in the Absence of a Host in a 6-inch Pot at 80°F.

Weeks elapsed	Pure Population		Field Soil	
	Numbers of <u>T. christiei</u> per 100 ml of soil	Percentage reduction	Numbers of <u>T. christiei</u> per 100 ml of soil	Percentage reduction
0	205 ^a /	0 %	69	0 %
2	196	3	66	3
4	157	23	47	32
8	140	32	42	40
12	115	44		
16	88	57		

a/ Average of two 100 ml samples.

T. christiei can adapt itself to survive at low soil moisture levels. No living specimens were found in soil with a moisture level of 0.9 per cent.

Laboratory Experiment 7. The green food dye proved most satisfactory for staining specimens of T. christiei, but the degree of staining varied with different individuals. The dye dispersed evenly throughout the specimens only after they were placed in a 1 per cent formalin solution. This stain was particularly successful in bringing out the reproductive system and cuticular structures.



Fig. 12 - Water mount of a live Trichodorus christiei recovered from soil with a moisture level of 1.7 per cent.

Among the cuticular structures not observed on unstained specimens were: (1) A series of dorsal and ventral pores spaced along the length of the body in the cuticle which appeared to be associated with a similar series of hypodermal cells; (2) two caudal pores which terminated within the cuticle; and (3) the posterior cephalids, completely encircling the cephalic region.

The Build-up of *Trichodorus christiei*.

The data presented above do not directly answer the question of what causes the build-up of T. christiei following fumigation; nevertheless, certain conclusions may be drawn. Indications are that predaceous nematodes play a minor role, as shown by the slow decline in high populations of T. christiei, in the absence of a host in the field. No statement can be made concerning possible effects of predaceous fungi.

There does not appear to be a stage in the life cycle of T. christiei resistant to D-D fumigation. Complete eradication of nematodes has seldom been obtained in the field with soil fumigation. At Sanford, Florida, the control of T. christiei is made more difficult due to its presence at relatively greater depths than other plant parasitic nematodes.

Crops on fumigated soil have improved growth over those on unfumigated soil, particularly during the seedling stages. This is due, in part, to the control of B. longicaudatus by fumigation, and as a result competition between this nematode and T. christiei is absent in fumigated soil. Under these conditions, T. christiei can attain a higher reproductive rate as shown by the greenhouse experiment presented above.

It is interesting to note that T. christiei reaches its reproductive potential (i.e., 12 progeny per 19 days at 80°F) at low levels of inoculum, but that population pressures are soon observed in 4-inch pots. The reason for this probably lies in the available food supply and its effect upon the reproductive rate. It is the considered opinion of the author that the crux of the problem of the build-up of T. christiei following fumigation is, in part, due to the increased number of available feeding sites.

SUMMARY

Population studies were made on the plant parasitic nematode, Trichodorus christiei, following field applications of D-D soil fumigant on fall, spring, and summer vegetable crops. Results showed that T. christiei could be found in greater numbers in fumigated soil than in unfumigated soil within 7 weeks after planting. Nevertheless, due to better seedling growth, the fumigated areas out-yielded those unfumigated. This build-up of T. christiei was not seen on those plots treated with the organo phosphate nematicide zinophos. This latter nematicide was also found to have a significant residual effect on subsequent crop yields.

Indications are that T. christiei does not possess a resistant stage to D-D fumigation. However, consideration should be given to the fact that at harvest there is a greater population of T. christiei at soil depths below 6 inches than above 6 inches.

The rate of mortality of T. christiei in the absence of a host, whether or not organic matter was incorporated, did not suggest the presence of a predator. Nor were populations of T. christiei affected by association with three free-living nematodes or an oligochaete in sterile soil.

Both T. christiei and Belonolaimus longicaudatus were found to parasitize pangolagrass. The effect of these two nematodes upon one another was studied when in combination on sweet corn. The numbers of

T. christiei were greatly reduced. Belonolaimus longicaudatus was found to be more pathogenic to sweet corn than T. christiei. In general the corn was damaged more by the combination of the species than by B. longicaudatus alone, though there were fewer numbers of B. longicaudatus in the combination.

The type of potting container was found to significantly affect the population numbers of T. christiei. In the greenhouse a glazed crock provided the best environment of those studied, while in a growth chamber a plastic container provided the best conditions.

A study of the reproduction of T. christiei under four constant temperatures, viz., 95°, 90°, 85° and 80°, showed the latter to be the most suitable. The effect of initial inoculum numbers on the reproductive rate of T. christiei was studied in sterile soil at 80°F. After 42 days the pots originally having 25 individuals produced increases of almost 3 times the rate of those with 100, and over 9 times the rate of those with 400, thus demonstrating the importance of population pressures as it is affected by the available food supply.

Single T. christiei specimens feeding on tomato seedlings growing at 80°F were found to give rise to an average of 12 progeny every 19 days. At this temperature the life cycle of T. christiei from egg to egg was found to be 17-18 days. Eclosion from the egg took place on the fourth day in the first larval stage which molted shortly thereafter. The third larval stage was first observed on the seventh day, after inoculating a gravid female onto tomato seedlings; the fourth larval stage, on the tenth day and the adult emerged after 2 weeks. The stage in the life cycle of T. christiei can best be judged by the size of the

gonadal primordium. Molting of T. christiei is described. This is the first record of a plant parasitic menatode not shedding its stylet, or any part of it, during molting.

Future work on the bionomics of T. christiei following fumigation should be directed towards the effects the improved host growth have on the reproduction of this nematode.

LITERATURE CITED

1. Alhassan, S. A., and J. P. Hollis. 1966. Parasitism of Trichodorus christiei on cotton seedlings. *Phytopathology.* 56: 573-574.
2. Allen, M. W. 1957. A review of the nematode genus Trichodorus with descriptions of ten new species. *Nematologica.* 2: 32-62.
3. Barker, K. R., and Gayle Worf. 1964. Parasitism of southern stock azaleas in Wisconsin by Tylenchorhynchus claytoni, Trichodorus christiei and Meloidogyne incognita. (Abstr.) *Phytopathology.* 54: 887.
4. Bird, G. W. 1966. Influence of host and geographic origin on populations of Trichodorus christiei. (Abstr.) *Nematologica.* 12: 88.
5. Chen, T. A., and W. F. Mai. 1965. The feeding of Trichodorus christiei on individually isolated corn root cells. (Abstr.) *Phytopathology.* 55: 128.
6. Chitwood, B. G., and B. A. Oteifa. 1952. Nematodes parasitic on plants. *Ann. Rev. Microbiol.* 6: 175-177.
7. Christie, J. R. 1959. Plant Nematodes Their Bionomics and Control. Gainesville, Fla. Univ. Fla. Press. 1-256.
8. Christie, J. R., and V. G. Perry. 1951. A root disease of plants caused by a nematode of the genus Trichodorus. *Science.* 113: 491-493.
9. Christie, J. R., and V. G. Perry. 1951a. Removing nematodes from soil. *Proc. Helminthol. Soc. Wash.* 3: 69-72.
10. Christie, J. R., and V. G. Perry. 1959. Mechanism of nematode injury to plants, p. 419-426. In C. S. Holton et al. (ed.), Plant Pathology Problems and Progress 1908-1958. Madison, Wisc.: Univ. Wisconsin Press.
11. Cobb, N. A. 1913. New nematode genera found inhabiting fresh water and non-brackish soils. *J. Wash. Acad. Sci.* 3: 432-444.
12. Esser, R. P., and E. K. Sobers. 1964. Natural enemies of nematodes. *Soil and Crop Sci. Soc. of Fla.* 24: 326-353.

13. Haasis, F. A., J. C. Wells, and C. J. Nusbaum. 1961. Plant parasitic nematodes associated with decline of woody ornamentals in North Carolina and their control by soil treatment. *Plant Disease Repr.* 45: 491-496.
14. Harrison, D. S., and R. E. Stall. 1961. Constant-temperature tanks for studying soil-borne diseases. *Trans. Am. Soc. Agr. Eng.* 1: 24-25.
15. Hoff, J. K., and W. F. Mai. 1964. Influence of soil depth and sampling date on population levels of Trichodorus christiei. *Phytopathology*. 54: 246.
16. Hutchinson, M. T., and H. T. Streu. 1960. Tardigrades attacking nematodes. *Nematologica*. 5: 149.
17. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Repr.* 48: 692.
18. Krusberg, L. R., and J. N. Sasser. 1956. Host-parasite relationship of the lance nematode in cotton roots. *Phytopathology*. 46: 505-510.
19. Lapage, G. 1935. The second ecdysis of infective nematode larvae. *Parasitology*. 27: 186-206.
20. Malek, R. B., W. R. Jenkins, and Ellen M. Powers. 1965. Effect of temperature on growth and reproduction of Criconemooides curvatum and Trichodorus christiei. (Abstr.) *Nematologica*. 11: 42.
21. Martin, G. C. 1966. Personal communication.
22. Micoletzky, H. 1922. Die freilebenden Erd-Nematoden. *Archiv. f. Naturgesch.* 87: 1-650.
23. Patrick, Z. A., R. M. Sayre, and H. J. Thorpe. 1965. Nematocidal substances selected for plant parasitic nematodes in extracts of decomposing rye. *Phytopathology*. 55: 702-704.
24. Perry, V. G. 1953. Return of nematodes following fumigation of Florida soils. *Proc. Fla. Hort. Soc.* 66: 112-114.
25. Rhoades, H. L. 1964. Effect of Crotalaria spectabilis and Sesbania exaltata on nematode populations and subsequent yield of snap beans and cabbage. *Proc. Fla. State Hort. Soc.* 77: 233-237.
26. Rhode, R. A., and W. R. Jenkins. 1957. Effect of temperature on the life cycle of the stubby-root nematode. (Abstr.) *Phytopathology*. 47: 29.

27. Rhode, R. A., and W. R. Jenkins. 1957a. Host range of Trichodorus sp. and its host-parasite relationship on tomato. Phytopathology. 47: 295-298.
28. Russell, C. C. 1962. The embryology and parasitic habit of Trichodorus christiei Allen with observations of predators. Unpublished Masters Thesis, Univ. Florida. Gainesville, Florida.
29. Russell, C. C., and V. G. Perry. 1966. Parasitic habit of Trichodorus christiei on wheat. Phytopathology. 56: 357-358.
30. Schaefferberg, B. 1950. Untersuchungen über die Bedeutung der Enchytraeiden als Humusbildner und Nematodenfeinde. Z. Pflanzenkrankh. Pflanzenschutz. 57: 183-191.
31. Thomason, I. J. 1959. Influence of soil texture on development of stubby-root nematode. (Abstr.) Phytopathology. 49: 552.
32. Thorne, G. 1932. Specimens of Mononchus acutus found to contain Trichodorus obtusus, Tylenchus robustus and Xiphinema americanum. (Abstr.) J. Parasitol. 19: 90.
33. Thorne, G. 1939. A monograph of the nematodes of the superfamily Dorylaimoidea. Capita Zool. 8: 1-190.
34. Thorne, G. 1961. Principles of Nematology. New York: McGraw Hill. 1-553.
35. Zuckerman, B. M. 1961. Parasitism and pathogenesis of the cultivated cranberry by some nematodes. Nematologica. 6: 135-143.

BIOGRAPHICAL SKETCH

Henry Vintcent Newton Morton was born on September 15, 1936, in Johannesburg, S. Africa. After attending school at St. Johns College for 10 years, he assumed the job of farm-manager on a dairy farm in Natal for a year before enrolling, in 1955, in the University of Natal at Pietermaritzburg.

In 1959, he received a Bachelor of Science in Agriculture with a major in Horticulture.

In 1960, he travelled to England where he joined the staff of Imperial Chemical Industries at Jealotts Hill Research Station, Berkshire, working with weedkillers.

In May, 1961, he immigrated to the United States by way of Canada and worked for Pulitzer Groves as grove foreman. In 1962, he became assistant to Dr. M. Cohen, Pathologist, at the Indian River Field laboratory, Fort Pierce, Florida.

In January, 1963, he enrolled in the Department of Fruit Crops at the University of Florida, where, with the aid of a Research Assistantship, he graduated with a degree of Master of Science in Agriculture in August, 1964.

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He returned to Gainesville to aid Professor Thorne in teaching the first course offered in Tropical Nematology at the University of Florida.

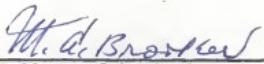
He completed the requirements leading to the Doctor of Philosophy Degree in June, 1967.

The author is a member of Alpha Zeta, Gamma Sigma Delta and Sigma Xi. In addition, he holds memberships in the Society of Nematologists, American Society of Horticulture and Florida State Horticultural Society.

In January, 1967, he married Virginia Ann Martin and adopted her two sons, Stephen and Scott.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June 20, 1967



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